Remarks

The Amendments to the Claims

Claims 66, 67, 81, and 88-95 have been amended to incorporate the recitations of originally filed claims 3 and 4. The amendments add no new matter.

The Objection to the Specification

The specification is objected to because the Accession numbers are missing on pages 2 and 6 [sic; 4]. The specification has been amended to insert the Accession numbers.

The Rejection of Claims 36-41, 66, 88, and 92 Under 35 U.S.C. § 102(a)

Claims 36-41, 66, 88, and 92 stand rejected under 35 U.S.C. § 102(a) over Prideaux *et al.*, WO 97/16531 ("Prideaux"). Applicants respectfully traverse the rejection.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. V. Union Oil Co. of California*, 814 F. 2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed Cir. 1987). Independent claims 66, 88, and 92 as amended recite a live *P. haemolytica* bacterium which (a) expresses no biologically active leukotoxin, (b) expresses a form of leukotoxin molecule which is a deletion mutant of about 66 kDa which lacks amino acids 34 to 378 and which induces antibodies which specifically bind to and neutralize biologically active leukotoxin, and (c) contains no non-*P. haemolytica* DNA.

Prideaux teaches modified microorganisms, including *P. haemolytica*, that produce a partially or fully inactivated leukotoxin. The inactivated leukotoxin, however, is not a

leukotoxin A deletion mutant of about 66 kDa which lacks amino acids 34 to 378, as recited in amended independent claims 66, 99, and 92. Rather, the leukotoxin in Prideaux's bacteria is inactive because the lktC gene has been inactivated and cannot carry out post-translational modification of the leukotoxin A protein. See page 4, lines 21-27:

> The present applicants have found that a microorganism which naturally produces an Lkt toxin may be engineered to produce an inactive Lkt toxin precursor by eliminating the post-translational activator of the precursor product. Accordingly, in a preferred embodiment the microorganism is unable to produce a post-translational activator of the Lkt toxin precursor or produces an inactivated post-translational activator of the Lkt toxin precursor. The post-translational activator may be a product of the Lkt C gene.

The Office Action specifically points to Prideaux's teaching "that the Lkt structural gene, i.e. LktA gene, may be partially or fully inactivated" and that this "includes an in-frame deletion of the gene." Office Action at page 9, citing claim 5 and page 7, lines 6-10 of Prideaux. The cited portions of Prideaux, however, do not teach or suggest making a bacterium that expresses the deletion form of leukotoxin A recited in each of the pending claims. Claim 5 of Prideaux merely recites that the partially or fully inactivated Lkt toxin is an unprocessed expression product of the LktA gene; it is the defective post-translational activator encoded by the LktC gene that causes the expression product of the LktA gene to be partially or fully inactivated. See the passage quoted above.

Similarly, the teaching at page 7, lines 6-10 does not teach a *Pasteurellaceae* bacterium that ***expresses*** the recited deletion form of leukotoxin A. The entire paragraph containing lines 6-10 of page 7 is reproduced below:

> Alternatively, the Lkt A gene product may be expressed entirely from an Lkt A gene or genes located on extrachromosomal

> elements such as plasmids. The Lkt A genes located on extrachromosomal elements may be expressed either in the presence or absence of selection for the extrachromosomal element. Thus, in one embodiment an extrachromosomal element containing an Lkt A gene may be introduced into a microorganism which lacks functional chromosomal Lkt A and Lkt C genes. The microorganism which lacks functional Lkt A and Lkt C genes may be produced by mutagenesis of the microorganism. The mutagenesis may result in deletion of the Lkt A and Lkt C genes or portions thereof.

This paragraph describes a microorganism that expresses no biologically active form of leukotoxin A: its own Lkt A gene does not encode a functional protein (the organism "lacks functional chromosomal Lkt A and Lkt C genes"), and the Lkt A gene product encoded on the extrachromosomal element is not functional because the microorganism does not contain a functional Lkt C gene (thus, the Lkt A protein cannot be post-translationally modified). However, this paragraph does not teach a microorganism that expresses a deletion mutant of about 66 kDa which lacks amino acids 34 to 378, as recited in the rejected claims.

Prideaux does not disclose the bacteria recited in claims 36-41, 66, 88, and 92 and thus does not anticipate the subject matter of those claims.

Applicants respectfully request withdrawal of the rejection.

The Rejection of Claims 36, 38-41, 66, 88, and 92 Under 35 U.S.C. § 103(a)

Claims 36, 38-41, 66, 88, and 92 stand rejected under 35 U.S.C. § 103(a) as obvious over Cruz *et al.*, *Mol. Microbiol. 4*, 1933-39, 1990 ("Cruz") in view of Briggs *et al.*, U.S. Patent 5,733,780 ("Briggs"). Applicants respectfully traverse the rejection.

The U.S. Patent and Trademark Office bears the initial burden of establishing a *prima facie* case of obviousness. The *prima facie* case requires three showings:

> First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Manual of Patent Examining Procedure, 8th ed., § 2142. Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in Applicants' specification. *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

The cited combination of art fails to meet this standard. First, the Office Action does not assert a legally sufficient motivation to have combined the teachings of Cruz and Briggs. Second, even if these teachings were *arguendo* combined, one of ordinary skill in the art would not have reasonably expected the mutant leukotoxin produced by the resulting bacterium to induce antibodies that specifically bind to and neutralize biologically active leukotoxin.

Cruz discloses plasmids derived from plasmid pYFC19. The modified plasmids contain a *P. haemolytica lktA* gene comprising an internal deletion. Cruz at page 1938, column 1, first full paragraph. Leukotoxin protein was expressed from the modified plasmids in an *E. coli* host (TB1). Paragraph spanning pages 1937 and 1938 and page 1938, column 2, paragraph 1. Cruz also discloses a *P. haemolytica* bacterium (biotype A, serotype 1) and expression of native leukotoxin from this bacterium. Cruz at page 1937, column 2, last paragraph, and page 1938, column 1, last paragraph. Native leukotoxin is by definition wild-type, *i.e.*, it does not comprise a mutation as recited in claims 36, 38-41, 66, 88, and 92. Cruz does not disclose a *P. haemolytica* bacterium which expresses an *lktA* gene comprising an internal deletion. Cruz does not teach or suggest that the proteins encoded by the disclosed *lktA* deletion mutants are

immunogenic or that they could induce neutralizing antibodies against biologically active leukotoxin.

Briggs teaches that a barrier to transformation of *P. haemolytica* can be overcome by treating DNA with a methylating enzyme, such as the *Pha*I methyltransferase. Such enzymes modify DNA substrates such that endonucleases which recognize 5'-GATGC-3' or 5'-GCATC-3' sequences are inhibited in their ability to digest such modified substrates. Col. 4, lines 21-27. Briggs teaches various methods that can be used to introduce mutations into *P. haemolytica*. *See, e.g.*, col. 4, line 47, to col. 5, line 33. In one method, methylated mutagenized DNA is introduced into *P. haemolytica*. Col. 4, line 66, to col. 5, line 4. In another method, unmethylated mutagenized DNA is introduced into a *P. haemolytica* bacterium that does not express *Pha*I restriction endonuclease. Col. 5, lines 4-9. Briggs also teaches a chimeric plasmid for unstable introduction of mutated DNA into *P. haemolytica*. Col. 5, lines 34-48.

Each of independent claims 66, 88, and 92 recites a live *P. haemolytica* bacterium with particular, recited characteristics:

> (a) the bacterium expresses no biologically active leukotoxin;
>
> (b) the bacterium expresses a form of leukotoxin molecule which is a deletion mutant of about 66 kDa which lacks amino acids 34 to 378 and which induces antibodies which specifically bind to and neutralize biologically active leukotoxin; and
>
> (c) the bacterium contains no non-*P. haemolytica* DNA.

The Office Action asserts it would have been *prima facie* obvious to one of ordinary skill at the time the present invention was made to "to use a *P. haemolytica* host cell in place of the *E. coli* host cell taught in Cruz because one of ordinary skill in the art would have a reasonable expectation that the use of a *P. haemolytica* cell which expresses an inactivated, yet antigenic

toxin would have produced a more efficient immune response due [to] the additional presence of other *P. haemolytica* antigens on the cell's surface." Page 13, last paragraph.

The asserted motivation is insufficient to meet the standard for establishing a *prima facie* case of obviousness. There is no teaching or suggestion in Cruz that the proteins encoded by the disclosed *lktA* deletion mutants are antigenic, as asserted in the Office Action, or that they could induce neutralizing antibodies against biologically active leukotoxin. The experiments reported in Cruz were performed *in vitro*, and no antibodies were produced. There is no teaching or suggestion at all in Cruz that the proteins encoded by the disclosed *lktA* deletion mutants are antigenic or that they would induce neutralizing antibodies against biologically active leukotoxin.

The leukotoxin molecules taught in Cruz lack regions of the leukotoxin protein that include a domain which is essential for lysis. See page 1936, column 1, lines 3-4. Cruz teaches that these mutant leukotoxin molecules protect cells against lysis by competitive binding to the cell surface in place of native leukotoxin. Page 1933, column 1, abstract, and page 1936, column 2, lines 6-9. Thus, the protective effect taught in Cruz does not occur via production of antibodies that specifically bind to and neutralize biologically active leukotoxin. If seeking to solve the problem of producing a vaccine to induce antibody-based immunity to pneumonic pasteurellosis in ruminants, the ordinary artisan would not have been motivated to construct a *P. haemolytica* bacterium comprising a mutant leukotoxin molecule which protects against lysis via competitive binding.

Moreover, none of the teachings of Cruz would have provided the ordinary artisan with a reasonable expectation of success that the disclosed leukotoxin molecules would be able to

induce the formation of inducing neutralizing antibodies against biologically active leukotoxin. Epitopes often are formed by amino acids from different portions of a protein that are placed in close proximity by protein folding. "[A]ntibodies raised against peptide fragments of a protein or against synthetic peptides corresponding to part of its sequence are ***occasionally*** found to bind to the native protein." Janeway *et al.*, IMMUNOBIOLOGY, 4th ed., 1999, at page 88, emphasis added (attached). The leukotoxin molecules taught in Cruz contain large deletions. See page 1934, figure 1. Thus, one of ordinary skill would not assume that antibodies raised against proteins missing such large portions of their structure to bind to the corresponding native protein.

Finally, even if the leukotoxin molecules of Cruz ***did*** induce antibodies, one of ordinary skill in the art could not have predicted with reasonable certainty that such antibodies would neutralize the biological activity of native leukotoxin. The antibodies recited in claims 36, 38-41, 66, 88, and 92 not only bind but also neutralize biologically active leukotoxin. It is well known in the art that not all antibodies that bind to a protein will neutralize that protein's biological activity. "Immune responses to infectious agents usually involve antibodies directed at multiple epitopes and only some of these antibodies confer protection." Janeway *et al.*, at page 562 (attached). One of ordinary skill in the art could not have predicted from the teachings of Cruz that molecules comprising such deletions would induce antibodies able to neutralize this biological activity. This teaching is found only in the present specification. It is improper for the Office Action to read the teachings of Applicants' specification into the prior art.

The Office Action has not asserted a sufficient motivation for the ordinary artisan to have combined the teachings of Cruz and Briggs. Even if, *arguendo*, these teachings were combined, the ordinary artisan would not have had a reasonable expectation of success that the mutant

leukotoxin molecules disclosed in Cruz could induce antibodies with the characteristics recited in the pending claims. The Office Action has therefore failed to make a *prima facie* case of obviousness based on the combination of Cruz and Briggs.

Applicant respectfully requests withdrawal of this rejection of claims 36, 38-41, 66, 88, and 92 under 35 U.S.C. § 103(a).

The Rejection of Claims 36, 38-41, 66, 88, and 92 Under 35 U.S.C. § 103(a)

Claims 36, 38-41, 66, 88, and 92 stand rejected under 35 U.S.C. § 103(a) as obvious over Potter *et al.*, U.S. Patent 5,422,110 ("Potter") in view of Briggs. Applicants respectfully traverse the rejection.

The Office Action has not made a *prima facie* case of obviousness over the combination of Potter and Briggs because those teachings, even if combined, do not teach or suggest all elements of the rejected claims.

The teachings of Briggs are summarized above. Potter discloses use of a leukotoxin as a carrier molecule to enhance the immune response to an antigen with which it is coupled in a chimeric (fusion) protein. Potter teaches that the leukotoxin portion of the chimeric protein increases the immunogenicity of the antigen to which it is coupled. See, for example, column 2, lines 44-50, and column 9, lines 10-15. Potter does not teach a form of leukotoxin protein that lacks amino acids 34 to 378 and which induces antibodies that specifically bind to and neutralize biologically active leukotoxin, as recited in amended claims 36, 38-41, 66, 88, and 92.

Even if *arguendo* the teachings of Potter and Briggs were combined, one could not produce a *P. haemolytica* bacterium that meet each element of the bacteria recited in claims 36,

38-41, 66, 88, and 92 because Potter does not teach the particular deletion mutant recited in those claims.

A *prima facie* case of obviousness over the combination of Potter and Briggs has not been made. Applicants respectfully request withdrawal of this rejection.

The Obviousness-Type Double Patenting Rejections of Claims 36-41 and 66-95

Claims 36-41 and 66-95 stand rejected under the judicially created doctrine of obviousness-type double patenting over claims 1-9 of U.S. Patent 6,495,145 and claims 22-29 of co-pending application Serial No. 09/736,169. Applicants will consider filing terminal disclaimers when the double patenting rejections are the only remaining rejections in this application.

The Rejection of Claims 36-41 and 66-95 Under 35 U.S.C. § 112, second paragraph

Claims 36-41 and 66-95 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Applicants respectfully traverse the rejection.

Claims 66, 67, 71, 81, and 88-95 are said to be vague and indefinite because they do not recite structural properties for the recited bacterium. Independent claims 66, 67, 81, and 88-95 have been amended to recite that the bacterium expresses a form of leukotoxin molecule "which is a deletion mutant of about 66 kDa which lacks amino acids 34 to 378." Thus the claims as amended recite structural properties.

Claims 66, 67, 81, and 88-95 are said to be vague and indefinite "because it is unclear how the antibodies neutralize biologically active leuktoxin." Page 3, first full paragraph. As

suggested in the Office Action, the claims have been amended to recite that the leukotoxin molecule "induces antibodies which specifically bind to and neutralize biologically active leukotoxin."

Claims 67-87, 89-91, and 93 are said to be vague and indefinite because they recite either lyophilized, killed, or lyophilized and reconstituted bacteria "wherein a live form of the lyophilized and reconstituted bacterium (a) expresses no biologically active leukotoxin . . . ." The Office Action correctly notes that lyophilized or killed bacteria do not actively express leukotoxin and requests clarification of how the claimed methods, vaccines, and feeds function. Because no leukotoxin protein are likely to be produced after the bacteria are lyophilized or killed, the recited bacteria must produce the recited leukotoxin before they are lyophilized or killed or after reconstitution.

Applicants respectfully request withdrawal of the rejection.

The Rejection of Claims 36-41 and 66-95 Under 35 U.S.C. § 112, first paragraph

Claims 36-41 and 66-95 stand rejected under 35 U.S.C. § 112, first paragraph, as not enabled. Applicants respectfully traverse the rejection.

The Office Action asserts that the specification does not enable any *P. haemolytica* bacterium that produces a leukotoxin molecule that is biologically inactive, induces antibodies which specifically bind to leukotoxin, and contains no foreign amino acid sequences. To advance prosecution, claims 36-41 and 66-95 have been amended to recite that the bacterium expresses a form of leukotoxin molecule that is a deletion mutant of about 66 kDa which lacks

amino acids 34 to 378. The Office Action acknowledges that such mutants are enabled. Page 4, paragraph no. 6.

The Office Action also asserts that claims 67-87, 89-91, and 93-95, which recite lyophilized bacteria, killed bacteria, or lyophilized and reconstituted bacteria, are not enabled because the mutant leukotoxin would not be expressed by such bacteria. In essence, the Office Action expresses doubt that vaccines or feeds comprising the lyophilized, killed, or lyophilized and reconstituted bacteria would work to induce immunity. To support a finding of non-enablement, the U.S. Patent and Trademark Office must establish a reasonable basis to question the enablement provided in the specification. *In re Wright*, 999 F.2d at 1562, 27 U.S.P.Q.2d (BNA) at 1513. The Office must not only explain why it doubts the statements in the specification's supporting disclosure, but also must support its assertions "with acceptable evidence or reasoning which is inconsistent with the contested statement." *In re Marzocchi*, 439 F.2d 220, 224, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971).

The specification teaches that lyophilized, killed, and lyophilized and reconstituted bacteria are suitable for use in vaccine formulations. See page 4, lines 14-15. The Office Action provides no reasonable basis to question this teaching.

The Office Action notes that the specification provides no working examples of immunity induced by such bacteria. The specification need not contain working examples if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 U.S.P.Q. (BNA) 642, 645 (C.C.P.A. 1970). The Office Action acknowledges that the specification enables live *P. haemolytica* bacteria that produce the recited mutant leukotoxin

protein, as well as feeds and vaccines comprising such bacteria. See page 4, paragraph no. 6. Methods of killing, lyophilizing, and reconstituting bacteria were well known in the art when this application was filed and need not be disclosed in the specification. Together with this knowledge in the art, the specification provides all the information needed to enable making and using the bacteria recited in claims 67-87, 89-91, and 93-95 as feeds and as vaccines.

The Office Action also asserts that "[t]he results from the use of the live bacterium do not correlate to the killed bacterium because the active agent is not expressed in the killed bacterium." Page 7. The Office Action cites no supporting evidence for this assertion. As taught in the specification and recited in claims 67-87, 89-91, and 93-95, live forms of the killed or lyophilized bacteria express the mutant leukotoxin protein before they are killed or lyophilized. Even though not actively expressed, the mutant leukotoxin protein would be present in the killed, lyophilized, and lyophilized and reconstituted bacteria. The Office Action cites no evidence why vaccines or feeds containing killed, lyophilized, or lyophilized and reconstituted bacteria containing the mutant protein would not function as taught in the specification.

The Office Action has not established a *prima facie* case that claims 67-87, 89-91, and 93-95 are not enabled. Applicants respectfully request withdrawal of the rejection.

Respectfully submitted,
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